DOI: 10.25205/978-5-4437-1691-6-213

BIVALENT ANTISENSE OLIGONUCLEOTIDES FOR INCREASED SELECTIVITY AND EFFICIENCY OF RNA DEGRADATION

V. O. Nnanyereugo[1], M. V. Dubovichenko[1], D. M. Kolpashchikov[2–4], A. A. Eldeeb[1]

[1] *SCAMT Institute, ITMO University, Saint Petersburg*
[2] *Chemistry Department, University of Central Florida, Orlando, USA*
[3] *Burnett School of Biomedical Sciences, University of Central Florida, Orlando, USA*
[4] *National Centre for Forensic Science, University of Central Florida, Orlando, USA*

✉ nnanyereugo@scamt-itmo.ru

Abstract

The efficiency of Antisense oligonucleotide (ASO) technology in vivo is limited by the affinity of relatively short (15–30 nucleotides) ASO that may fail to bind RNA folded in stable secondary structure. We present a design, having two short sequences, that bind RNA targets across an extended region of their folded structures, increasing RNA cleavage efficiency and maintaining cleavage specificity.

Multivalent recognition and binding of biological molecules is a natural phenomenon that increases binding stability (avidity) without decreasing the recognition specificity [1]. The advantage of this approach has been underexplored in mRNA recognition by ASOs. ASO technology uses oligonucleotides that bind to targeted RNA via Watson-Crick base pairing, causing RNase-H dependent degradation and suppressing its biological function. Despite their potential as therapeutic agents, ASOs face challenges in selectivity and efficiency which limits their clinical application [2].

Longer ASO sequences can address the problem of short ASOs that may fail to bind RNA folded in stable secondary structure, but it compromises specificity of RNA binding. To address the specificity problem resulting from using longer ASO sequences, three bivalent variants were designed and analyzed. BivASO; with longer sequences binding to RNA conventionally, BivASO(cis); with short ASO sequences binding RNA conventionally, and BivASO(trans) with short ASO sequences binding to RNA across a region of its folded secondary structure (Fig. 1B). We hypothesize that hybridization of bivalent ASO with shorter sequences to RNA across a region of its folded structure (trans BivASO) provides efficient and specific RNA cleavage in comparison to ASOs with conventional design.

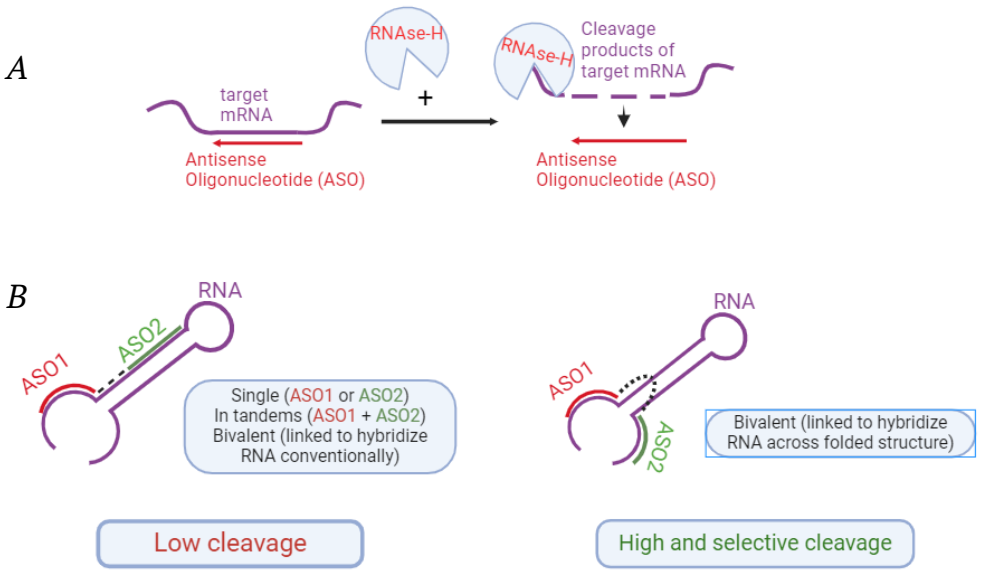

Fig. 1. Showing (*A*) principle of the conventional ASO technology; (*B*) Benefit of the Bivalent ASO trans variant as compared to single ASOs or tandems

Single oligonucleotide sequences and bivalent ASOs were made to target the sequence of RNA-58, a fragment of strA gene, coding the protein responsible for streptomycin resistance [3]. RNA-58 had folding energy of −24.50 kcal/mol [4]. RNA 58 was incubated with each ASO, individually and in tandems, to cooperatively and independently bind to RNA-58 and trigger RNase H dependent cleavage. Apart from BivASO cis, the multivalent association generally showed improved cleavage efficiency when compared to their usage individually or in tandems. BivASO and BivASO(trans) demonstrated approximately 50 and 31% cleavage efficiency, respectively (Fig. 2). This difference can be attributed to the longer target binding sequence of BivASO, which enables tighter binding. The variants were further analyzed incorporating mismatched bases in the sequence design. The BivASO(trans) demonstrated better specificity as compared to the other two variants. This difference can be attributed to the shorter arms of the trans version as longer nucleotide sequences are more tolerant to mismatches.

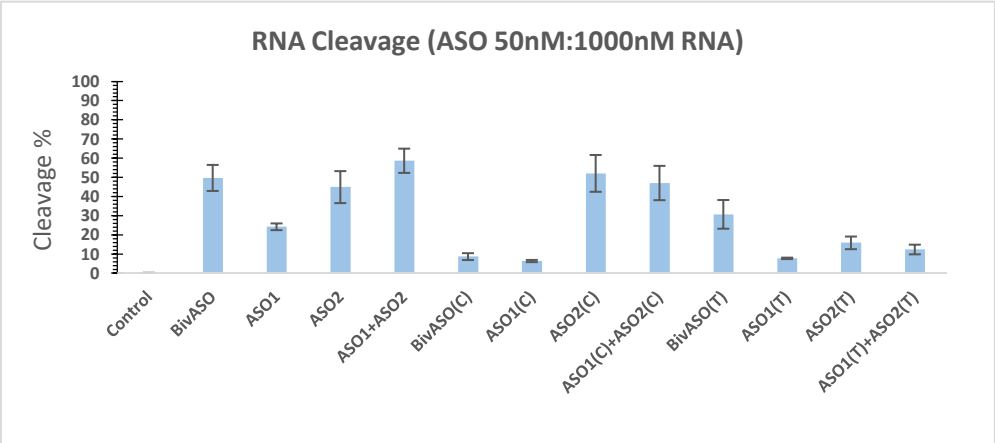

Fig. 2. Graph showing RNase H dependent RNA cleavage in the presence of various ASO agents at 37 °C for 20 minutes. Buffer solution (1 mM MgCl2+, 20 mM NaCl, 50 mM HEPES, and 150 mM KCl). 20 % Urea PAGE at 80V for 150 minutes. The Data are averaged from three independent measurements. Error bars represent 3 standard deviations from the average

In conclusion, improved efficiency from the trans variant shows great prospect in overcoming the low efficacy and selectivity associated with the use of ASOs in clinical applications.

References

1. Dubovichenko M. V. et al. Multivalent DNAzyme agents for cleaving folded RNA // Nucleic Acids Res. 2024. Vol. 52, No. 10. P. 5866–5879.

2. Drozd V. S. et al. Binary Antisense Oligonucleotide Agent for Cancer Marker-Dependent Degradation of Targeted RNA // Nucleic Acid Ther., 2022. Vol. 32, No. 5. P. 412–420.

3. Sundin G. W and Bender C. L. Dissemination of the strA-strB streptomycin-resistance genes among commensal and pathogenic bacteria from humans, animals, and plants // Mol. Ecol. 1996. Vol. 5, No. 1. P. 133–143.

4. Zuker M. Mfold web server for nucleic acid folding and hybridization prediction // Nucleic Acids Res. 2003. Vol. 31, No. 13.